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# Sphingosine-1-phosphate Dysregulation And The Development Of Pelvic Organ Prolapse (Poster)

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# Sphingosine-1-Phosphate Dysregulation and the Development of Pelvic Organ Prolapse

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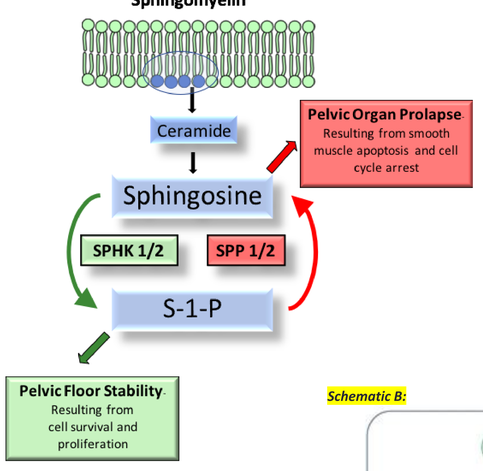
## INTRODUCTION & OBJECTIVES

- **Pelvic Organ Prolapse (POP**) is a disorder that occurs when the musculature of the pelvic floor weakens, resulting in pathologic descent of pelvic organs into the vaginal canal.
- While risk factors for POP have been suggested such as genetic predisposition, childbirth, obesity, and advancing age, the etiology of this condition remains largely unknown.
- Females have an 11% lifetime risk of POP which can result in urinary, bowel, and sexual dysfunction that can significantly impair a woman's quality of life.[1]
- Sphingosine-1-Phosphate (S1P) is a bioactive lysosphingolipid with countless metabolic functions including regulation of cell proliferation and smooth muscle (SM) contractility.[2] S1P is generated by the phosphorylation of sphingosine by sphingosine kinase, which exists as two major isoforms (SPHK1 & SPHK2).
- We hypothesized that SPHK isoform expression could be altered in vaginal SM, thereby leading to decreased vaginal wall stability and subsequent POP.

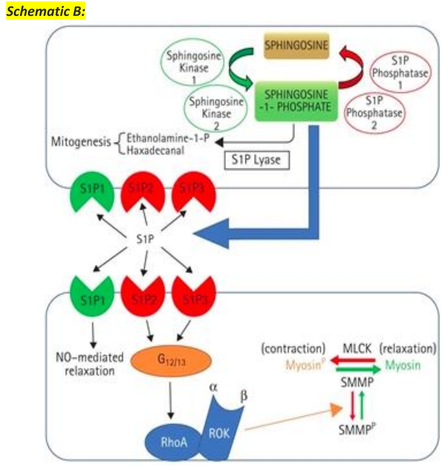
## BACKGROUND

***Schematic A:***




***Schematic A:*** Demonstrating the biosynthetic pathway of pro-apoptotic sphingosine, which is implicated in the development of POP, and anti-apoptotic S1P, which is thought to stabilize smooth muscle by promoting growth and proliferation.

***Schematic B:***




***Schematic B:*** [3]
Representation of the S1P pathway. Binding of S1P to its receptors (S1P2, S1P3) results in smooth muscle contraction.

## METHODS

- Anterior vaginal wall smooth muscle and epithelial tissue samples were obtained from women undergoing surgery at Cooper University Hospital
- **Control Samples:** obtained from women undergoing routine hysterectomy with no history of POP
- **Experimental Samples**: obtained from women with POP undergoing reconstructive surgical repair
- Tissue samples were extracted into SDS-PAGE sample buffer. Quantitative Western Blot analyses using Bio-Rad's No-Stain Gel line/Trans-Blot Turbo Blotting System/ChemiDoc Imager/Image Lab 6.0 software and fluorescently tagged antibodies were used to determine relative expressions of SPHK1, SPHK2, & SPP1.
- Total Protein Normalization **(Fig. A)**: Relative expression of target protein to total lane protein visible on ultraviolet light-activated no-stain was determined which minimized the effects of unequal loading between lanes.

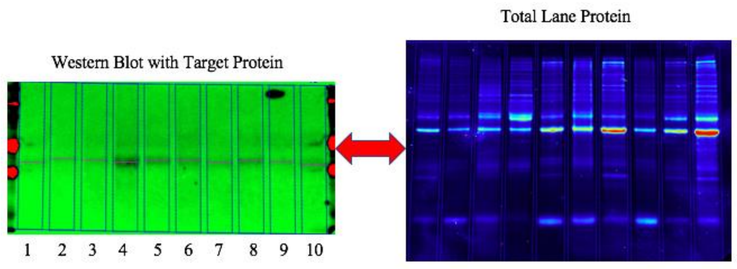



**Figure A**: Demonstrating the process of total protein normalization.

## RESULTS

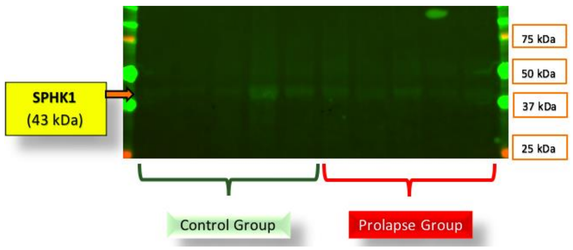



**Figure 1**: **Vaginal Smooth Muscle Tissue**

- SPHK1 expression 46% lower in POP compared to controls normalized to total protein

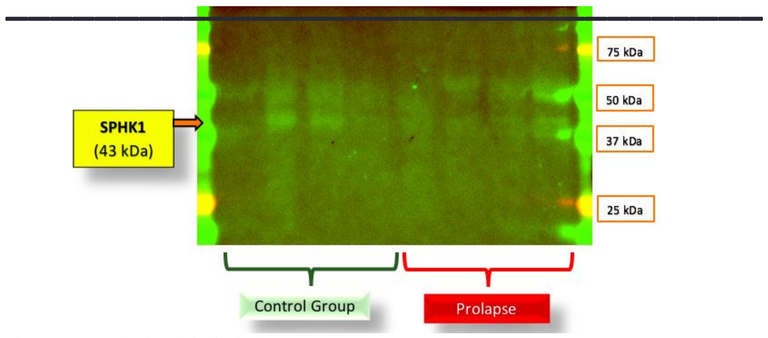



**Figure 2**: **Vaginal Epithelial Tissue**

- SPHK1 expression 40% lower in POP compared to controls normalized to total protein

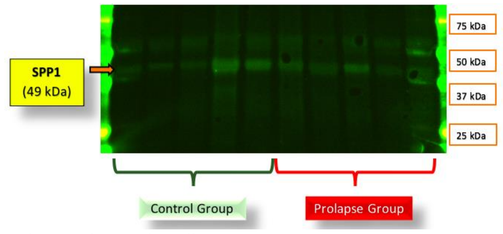



**Figure 3**: **Vaginal Smooth Muscle Cells**

- SPP1 expression 13% lower in POP compared to controls normalized to total protein

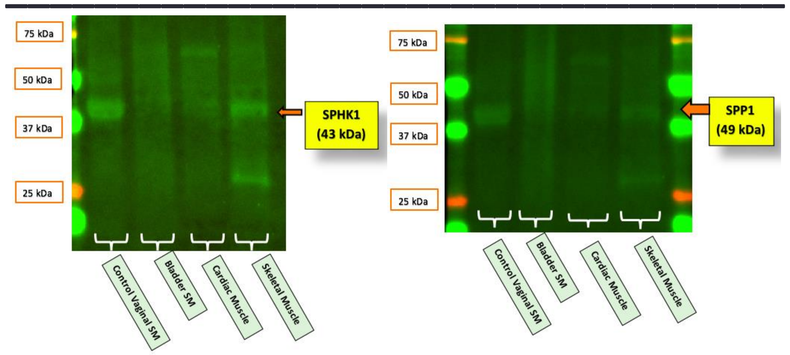



**Figure 4: Human Tissue Extracts.**
12.5 Demonstrates the presence of SPHK1 (Left) and SPP1 (Right) in human vaginal, cardiac, and skeletal muscle. The presence of these enzymes in human bladder samples was inconclusive.

***Additional Blots not shown:***

- Using the same exact SM and epithelial samples under identical conditions, we could not identify SPHK2 in either control or POP samples.
- We could not identify SPP1 in epithelial samples.

## DISCUSSION & CONCLUSIONS

- Our novel data suggests that SPHK1 is the predominant sphingosine kinase in human anterior vaginal SM and epithelial tissue samples and that its levels are decreased dramatically in women with POP compared to controls.
- Levels of SPP1 were also found to be lower in vaginal wall smooth muscle (but only slightly). The larger decrease in SPHK1 vs SPP1 expression would suggest a shift toward a higher sphingosine-to-S1P ratio and thus a shift toward apoptosis and cell cycle arrest and overall decreased vaginal wall stability.
- These findings suggest a POP preventative treatment strategy of stabilizing SPHK1 levels in the vagina.
- Our study utilized human samples making our findings translationally relevant.
- A limitation of our data is that only 5 control and 5 POP human samples were utilized. More samples will be analyzed in the future.

## REFERENCES

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3.) Aydin M, Downing K, Villegas G, Zhang X, Chua R, Melman A, DiSanto ME. The sphingosine-1-phosphate pathway is upregulated in response to partial urethral obstruction in male rats and activates RhoA/Rho-kinase signalling. BJU Int. 2010;106(4):562-71.